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LIFE HISTORY OF *CYBOCEPHALUS NIPPONICUS* ENDRÖDY-YOUNGA (COLEOPTERA: CYBOCEPHALIDAE), A PREDATOR OF *AULACASPIS YASUMATSUI* TAKAGI (HEMIPTERA: DIASPIDIDAE)

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Abstract.—The life history of the predatory beetle *Cybocephalus nipponicus* Endrödy-Younga was studied by rearing the beetle on the cycad aulacaspis scale, *Aulacaspis yasumatsui* Takagi. Mean development times of egg (7.3 ± 0.8 days), larval (13.7 ± 1.1 days), and pupal (18.6 ± 1.6 days) stages were determined. Mortality in each life stage, adult longevity, and adult sex ratios also were measured. A clarification of differences between *C. nipponicus* and *C. binotatus* is included.

Key Words: Beetles, biological control, life cycle, longevity, scale predator, cycads

Cybocephalids are one of the most economically important groups of natural enemies against scale insects (Alvarez and Van Driesche 1998a). Larvae and adults are voracious predators and have many desirable traits for use as biological control agents. *Cybocephalus nipponicus* Endrödy-Younga was released and later established in the Washington D.C./Maryland area to combat the euonymus scale, *Unaspis euonymi* (Comstock) (Drea and Carlson 1988, Drea and Henrickson 1988). Alvarez and Van Driesche (1998a) later released and established populations of *C. nipponicus* in New England. This same species also was released, under the false identification of *Cybocephalus binotatus* Grouvelle, into the Miami area in 1998 for control of the cycad aulacaspis scale, *Aulacaspis yasumatsui* Takagi (Anonymous 1998, Howard et al. 1999, Howard and Weissling 1999). While *C. nipponicus* and *C. binotatus* appear similar, they each have very distinctive male genitalia (Endrödy-Younga 1971) and *C. binotatus* (Fig. 1A) has two large black spots on the pronotum, which are absent in *C. nipponicus* (Fig. 1B). Although *C. nipponicus* previously was established in Florida before 1998 (according to specimen label data in the Florida State Collection of Arthropods), its range and abundance in the state before 1998 is unknown (Smith and Cave, in press).

The cycad aulacaspis scale (CAS) is the most damaging scale found on cycads in Florida (Hodges et al. 2003). CAS is native to Thailand but is found throughout China and southeastern Asia, as well as on several Caribbean islands, Florida, and Hawaii (Ben-Dov et al. 2003). In 1992, CAS had become such a problem in Hong Kong that 70–100% mortality was recorded in infested king sagos, *Cycas revoluta* Thunberg (Hodgson and Martin 2001). The first detection of CAS in Florida occurred

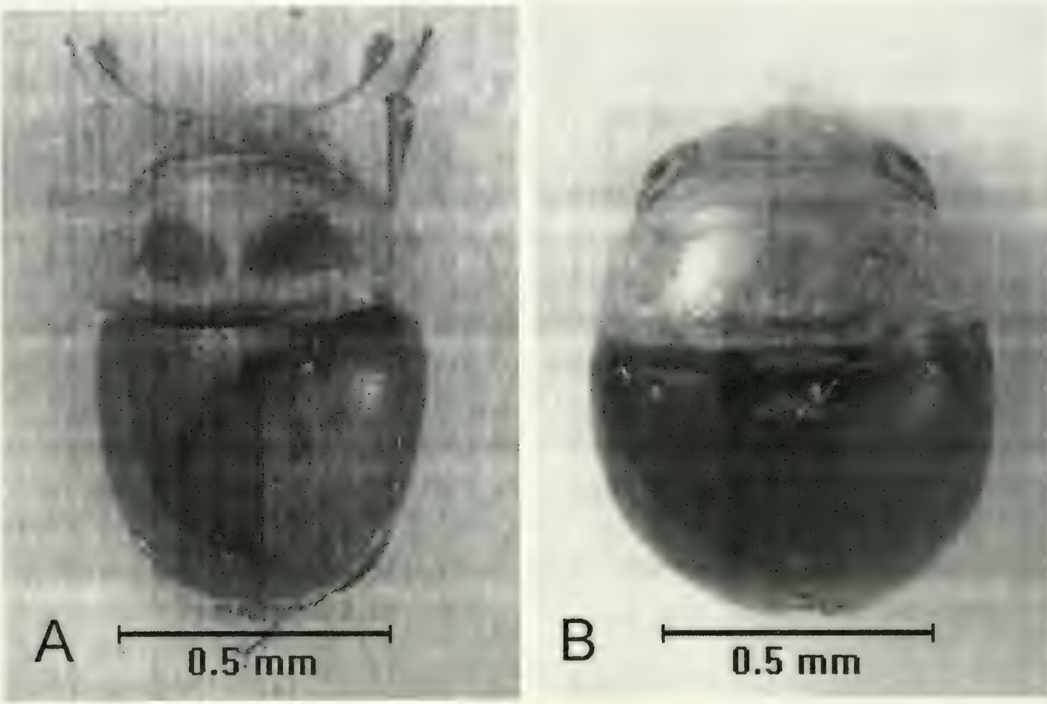


Fig. 1. Dorsal habitus. A, *Cybocephalus binotatus*. B, *C. nipponicus*.

in 1996 in Miami at the Montgomery Botanical Center. The scale was thought to have arrived on infested cycads imported from southeastern Asia (Howard and Weissling 1999). By the end of 1997, CAS had spread throughout Miami and as far north as Lake Okeechobee and could be found on 20 species of cycads (Howard and Weissling 1999), however it seems to prefer *Cycas* and *Stangeria* (Emshousen, personal communication, 2004). This led to the spread of CAS to Hawaii in 1998 through the legal importation of infested cycads from Florida (Hodgson and Martin 2001). At present, CAS has been reported from Pensacola east to Jacksonville and south into the Florida Keys. Infested cycads in northern Florida were suspected transplants from southern nurseries rather than natural progression of the scale northward.

The objective of this study is to collect life history date on *C. nipponicus* using CAS as prey, and compare these to the results from Tanaka and Inoue (1980) and Alvarez and Van Driesche (1998a) using euonymus scale as prey. A better understanding of these beetles using different prey will lead to greater understanding of how they perform as biological control agents in the field, and consequently increase success in controlling CAS.

Materials and Methods

A colony of CAS was reared on king sagos in a sealed greenhouse to keep out possible predators and/or parasitoids. Small king sagos were infested with CAS by placing large numbers (>100) of eggs on each plant. Once infested, plants were then placed in contact with other non-infested plants to spread non-parasitized scales to other plants. Thus, we could be confident that parasitoids and predators did not invade the colony.

A colony of *C. nipponicus* was initiated from individuals collected in south Miami (25°38′21″N, 80°20′09″W). *Aphanogmus albicoxalis* Evans and Dessart (Hymenoptera: Ceraphronidae) parasitizes the pupae of *C. nipponicus* in southern Florida (Evans et al. 2005), but this parasitoid was excluded by collecting only adult beetles and subsequently rearing future generations in sealed cages (0.5 m × 0.5 m × 0.5 m). Cycad leaves infested with CAS were cut from the colony plant, with rachis bases placed in floral water tubes for hydration, and then placed in rearing cages. The leaves and interior of cage were misted with distilled water every three days. A moist sponge also was provided for hydration. Leaves were replaced every three weeks. Old leaves were held in separate cages for three weeks to recover emerging beetles. Beetle and scale voucher specimens were placed in the Florida State Collection of Arthropods (FSCA).

All life cycle studies were carried out in temperature- and humidity-controlled cabinets set at 25°C with a relative humidity of 80% and a photoperiod of 14:10 (L:D). Each treatment was initiated by isolating 25–30 mating pairs of beetles randomly selected from the laboratory colony. Each pair was placed in a 25-dram plastic vial with one *C. revoluta* leaflet infested with male and female CAS. After 24 hours the beetles were removed and eggs collected from beneath the scale armor on the leaflet. Eggs were measured (length and width), and then, to simulate natural conditions, placed on the surface of a small, clean piece of *C. revoluta* leaflet and covered with the armor of an adult female CAS. The leaflet piece was placed in the well of a rectangular tissue culture dish, covered with parafilm, and placed in the environmental chamber. Eggs were checked daily until larvae emerged.

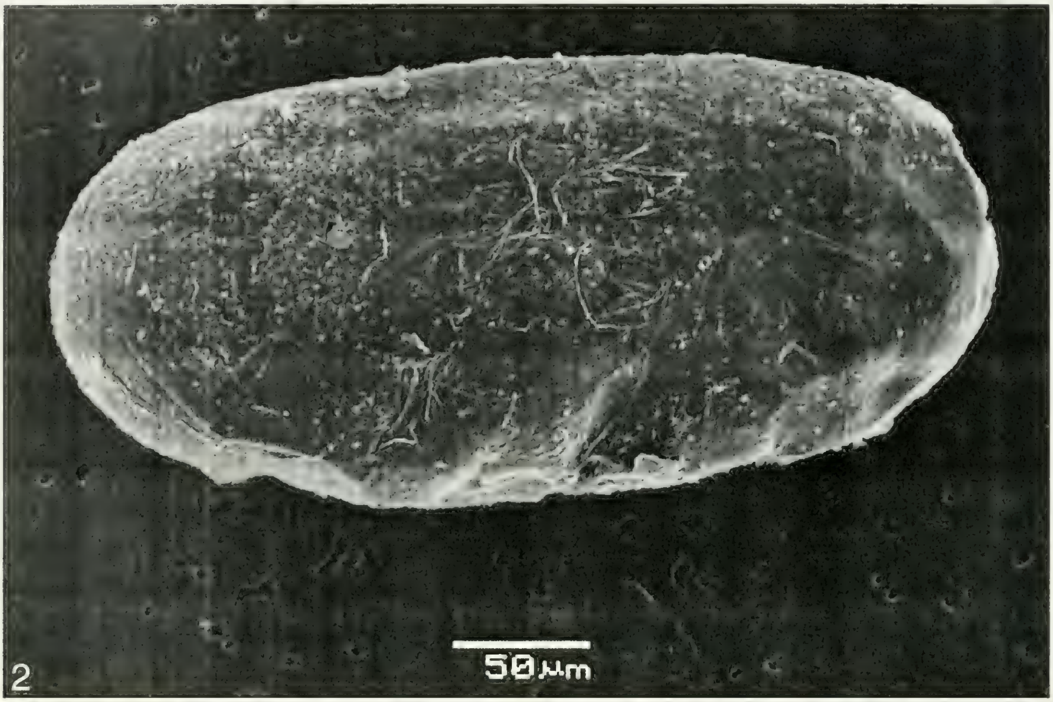
Newly emerged larvae were placed in 25-dram plastic vials with freshly-cut, CAS-infested leaflets on top of sterilized sand in the bottom of the vial. Old leaflets were replaced with fresh, infested leaflets every five days throughout the life cycle. A fine mesh cloth was placed over the top of the vials to allow airflow. Larval and pupal development was checked daily. Upon emergence from the pupal case the adults remained in plastic vials and were provided with 20–30 fresh CAS every 3 days. Adults were checked daily until death.

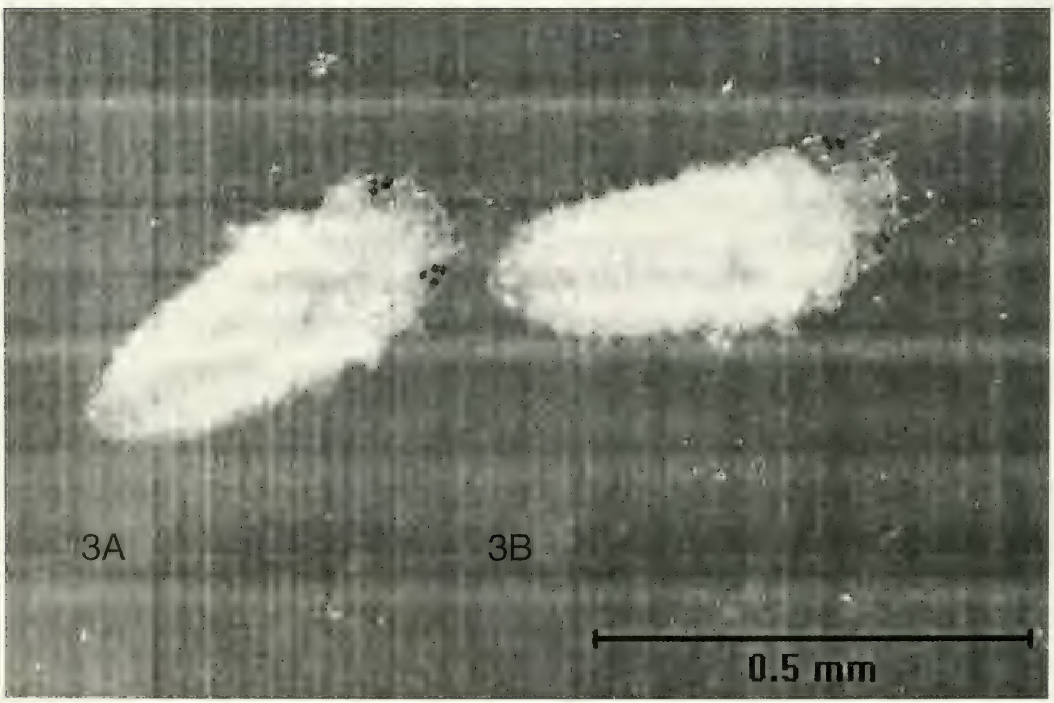
Eggs, larvae, and pupae were critical-point dried in a Tousimis®samdri-780 A, and sputter coated with a gold-palladium alloy. Images were taken with a JEOL® JSM-5510LV scanning electron microscope and a Syncroscopy® automontage photography system.

Descriptive statistics were generated in SAS (2001) using a PROC UNIVARIATE analysis. The dependent variable was number of days in each stage and the independent variable was the stage itself. PROC UNIVARIATE and PROC *t*-test were used to generate statistics for adult longevity.

Results and Discussion

The egg of *C. nipponicus* is elongate oval with both ends rounded, relatively large measuring 0.42 mm by 0.20 mm (n = 50) and usually light gray to purple. Eggs were usually found singly inside the vacated tubular cover of a male scale or under the armor of a female scale, usually with a live scale beneath but occasionally with a dead female. During low scale density, as many as 5 eggs under one female armor were observed. An egg deposited within the male scale cover fit snugly and had almost the same diameter as the scale cover, thereby allowing only one egg to be placed in one male cover. The surface was smooth aside from debris sticking to the surface (Fig. 2) and was slightly tacky, allowing the egg to stick to the substrate. Females typically laid about 3 eggs per day and





Figs. 2–3. *Cybocephalus nipponicus.* 2, Egg. 3, First-instar larva dorsal habitus (A), ventral habitus (B).

Table 1. Development of *Cybocephalus nipponicus* on *Aulacaspis yasumatsui.*

Stage	Mean (Days)	SE	Range (min-max) (Days)	N
Egg	7.3	0.1	4 (5–9)	131
Larva	13.7	0.1	4 (12–16)	94
Pupa	18.6	0.2	6 (16–22)	44
Total development time	39.5			
Male life-span	89.1	9.7	146 (9–155)	23
Female life-span	110.0	9.6	175 (15–190)	31

on average 288 eggs in a lifetime (Alvarez and Van Driesche 1998a). Eggs hatched about 7 days after oviposition (Table 1). Eyespots could be seen 1–2 days before larval emergence.

When the larva emerged, the chorion split along the longitudinal axis and the larva wriggled free. This process took about 15 minutes compared to the 30 to 45 minutes reported by Blumberg and Swirski (1982) on the life history of *Cybocephalus micans* Reitter and *Cybocephalus nigriceps nigriceps* (J. Sahlbery). The neonate larvae were white or yellowish with long setae along the body, but after feeding for a day turned light purple or lavender, with 4 black stemmata on each side of the head (Figs. 3A, B). Not only were larvae covered in long, slender setae but also shorter trumpet-shaped setae (Fig. 4). After emergence, larvae immediately began to feed either on the scale eggs sharing the space beneath the armor or rarely on the female scale. If an egg hatched in a male scale cover, the larva would go to the nearest food source. Larvae continued to move from scale to scale feeding on males, females, and eggs but spent the most time underneath female armor. They also were seen cannibalizing other larvae when scale density was extremely low, as mentioned by Alvarez and Van Driesche (1998a). Larvae fed for 9 to 10 days.

Three instars (Figs. 5A, B) were observed, similar to *C. micans* and *C. n. nigriceps* (Blumberg and Swirski 1982). However, Ahmad (1970) recorded four instars in *Cybocephalus semiflavus* Champion. When molting, the cuticle ruptured along the top of the head capsule and along the median dorsal region of the body. The larva emerged from the anterior portion of the old exuviae first, then wriggled vigorously to extract the posterior body portion. The posterior portion of the old exuviae remained attached to the substrate. Average larval development was about 14 days (Table 1).

If bright light was shone on larvae they immediately moved underneath a scale armor or debris. Once disturbed larvae raised the head and body away from the leaf surface, arching the body into a C-shape, and holding to the substrate with the conical protuberance found on segments 8 and 9. This threat posture is similar to that used by the larvae to extract themselves from old exuviae.

Larvae became less active 2 to 3 days prior to pupation, stopped feeding, and eventually becoming immobile and attaching the posterior of the abdomen to the substrate before forming the pupal case. Larvae gathered pieces of scale armor and incorporated these pieces into an ovoid pupal chamber with one end flatter where it attached to the substrate (Fig. 6A).

Pupal chambers often were found in the anterior portion between leaflet and leaf rachis or near the leaflet base. However, pupal chambers also were observed along leaflet and rachis. When not given access to scales, larvae used

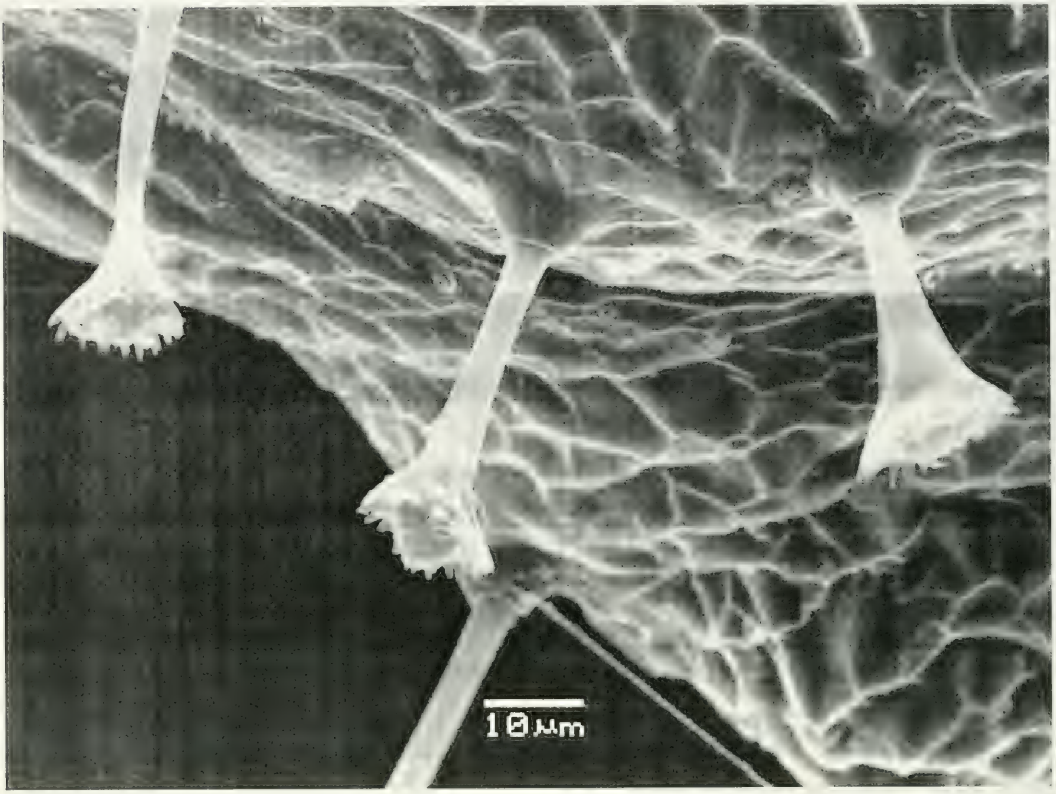
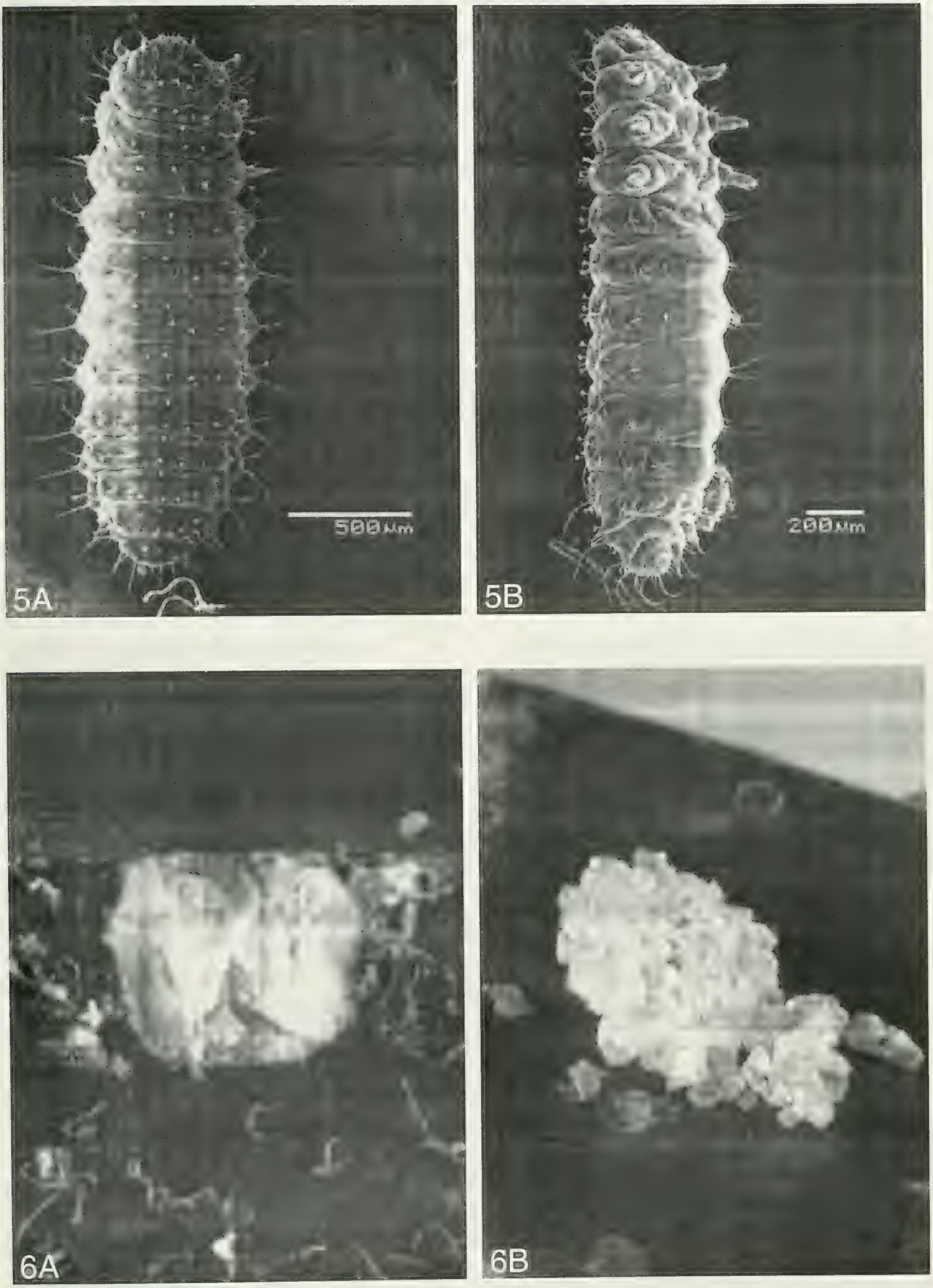


Fig. 4. Short trumpet-shaped setae on third-instar larva of *Cybocephalus nipponicus*.

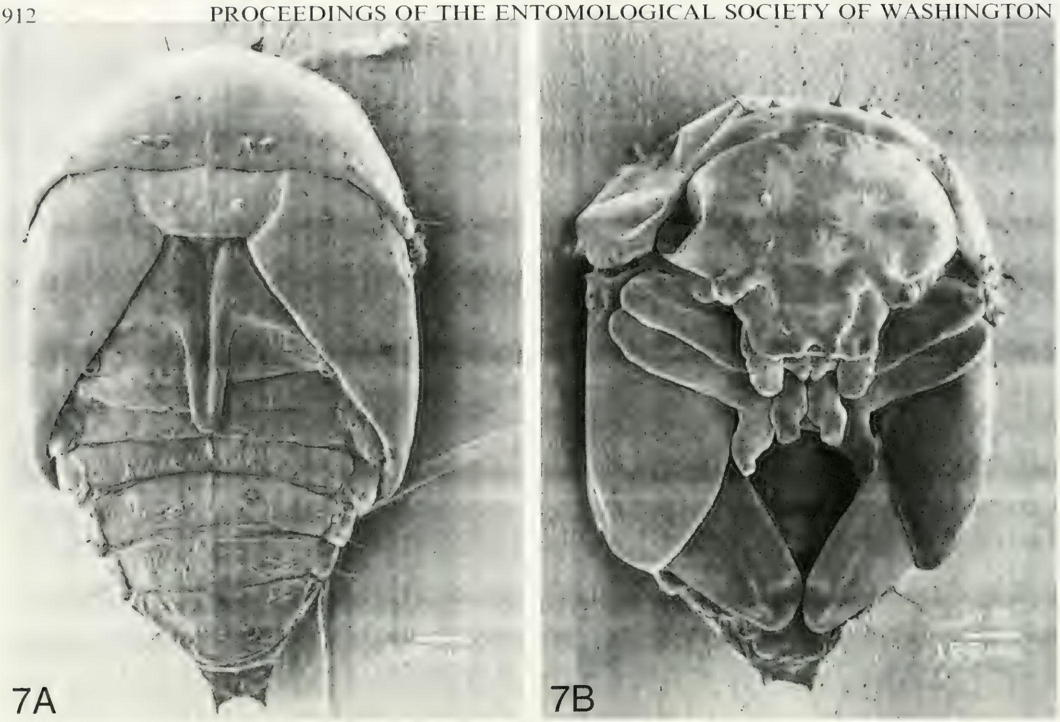
sand or other organic material to construct the pupal chamber (Fig. 6B), sometimes attaching to the leaf or dropping to the sand. Infrequently, larvae dropped to the sand and made a sand cocoon even when scales were available. This behavior is common in *Cybocephalus* and has been suggested or recorded for other species (Clausen and Berry 1932, Flanders 1934, Smirnoff 1954, Blumberg and Swirski 1982). All pupae exhibited typical exarate features (Figs. 7A, B). Pupation lasted about 18 days (Table 1). Beetles exited the chamber by chewing an emergence hole.

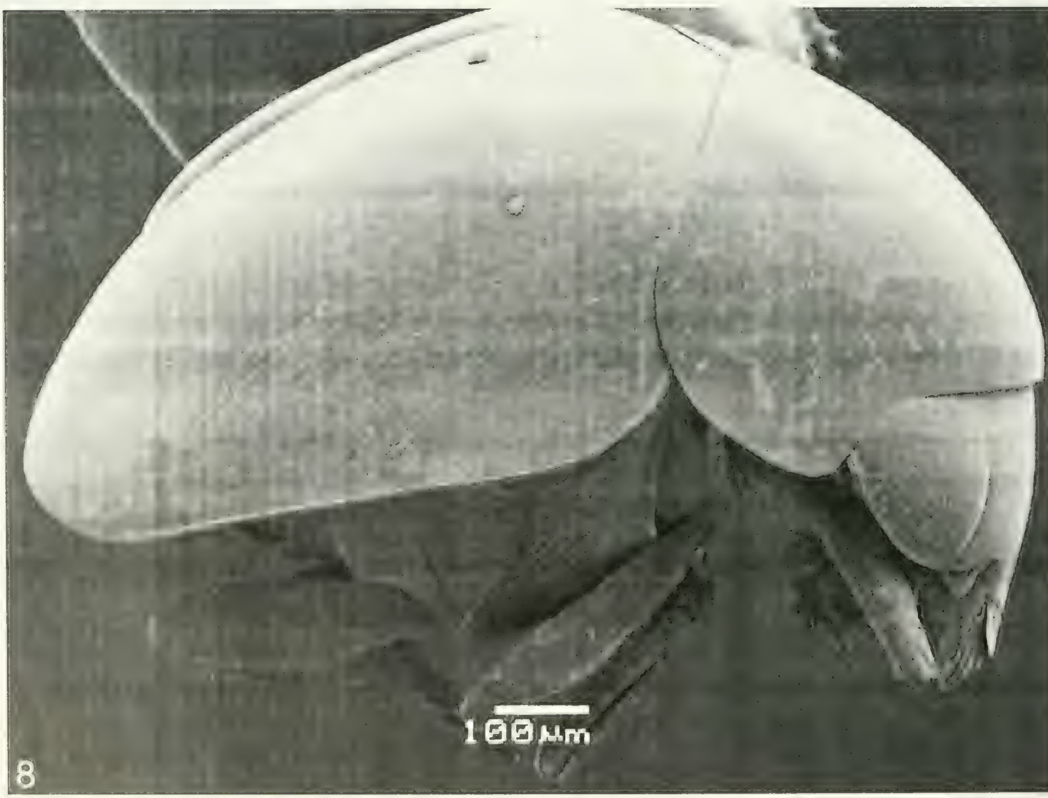
There was no significant difference in development time from egg to adult between sexes (t = 1.50, df = 52, P = 0.1389). Total development from egg to adult lasted about 40 days (Table 1), thus it is conceivable that 7–8 generations could be produced per year in southern Florida or other areas with amenable temperatures. Alvarez and Van Driesche (1998a) suggested that these beetles were capable of producing 3 generations per year in New England.

Preoviposition lasted about 4 days (n = 29) with some females laying eggs as early as 2 days (n = 2) after emergence. Adults (Fig. 8) began feeding soon after emergence and consumed an average of 4 scales per day, with females typically eating more than the males. Disproportionate feeding is probably due to size, because males are smaller than the females. Maximum female longevity was 190 days, with an average of about 110 days (Table 1). Average longevity of males was 89 days (Table 1) with a maximum of 155 days. Due to high variation, a t-test showed no significant



Figs. 5–6. *Cybocephalus nipponicus.* 5, Third-instar larva dorsal habitus (A), ventral oblique habitus (B). 6, Pupal chamber made from female scale covers (A), chamber made from sand (B).





Figs. 7–8. *Cybocephalus nipponicus*. 7, Pupa dorsal habitus (A), ventral habitus (B). 8, Adult, lateral habitus.

Table 2. Mortality (%) of immature stages of *Cybocephalus nipponicus* reared on *Aulacaspis yasumatsui* (n=200).

Stage (x)	No. Alive at Start (l_x)	No. Dying in Stage (d_x)	Apparent Mortality (q_x)
Egg	200	44	0.22
Larva	156	134	0.86
Pupa	22	2	0.09
Adult	20		

difference between longevity of the sexes (t = 1.50, df = 52, P = 0.1389). These findings are intermediate between the 78 days for males and 99 days for females reported by Alvarez and Van Driesche (1998a) at 22°C and 122 days for males and 143 days for females reported by Tanaka and Inoue (1980), who do not detail their experimental temperatures used. Shorter male longevity may be due to their more active nature. Males were observed moving around the cages more often than females. Often, 5 or 6 males would chase a female for hours before finding a new female to pursue. The sex ratio of emerging adults was 23:31 (male:female).

Our results were similar to those of Alvarez and Van Driesche (1998a) and Tanaka and Inoue (1980). However, all stages developed slower in the former study (9.1 days for eggs, 14.5 days for larvae, and 20.4 days for pupae) in comparison to our results, but this may be due to their lower experimental temperatures or the food source. Population differences may account for some discrepancies in the life history parameters of the beetles in each study. The beetles studied by Tanaka and Inoue (1980) originated in Japan and those studied by Alvarez and Van Driesche (1998a) originated in Beijing, China. Our beetles may have originated in Thailand, however, this is only speculation considering the beetles were present in Florida before the recorded introduction in 1998 (Smith and Cave, in press). Mortality rates in our study also were slightly higher, possibly due to high humidity. Fungal growth was a consistent problem in the humid environment of our rearing chambers. Scales often became so encrusted on the cycad leaflet that saprophytic fungi quickly spread. Alvarez and Van Driesche (1998a) did not record relative humidity, thus a comparison cannot be made.

Mortality was highest during the larval stage. The first few days of larval development proved to be most difficult (Fig. 9). Eggs and pupae seemed to be quite hardy with mortality of 22% and 9%, respectively (Table 2), which are similar to the 14% and 8% mortality rates found by Alvarez and Van Driesche (1998a). Therefore, the 86% mortality rate in the larval stage (Table 2) is interesting and not predicted. However, when reared on San Jose scale, *Quadraspidiotus perniciosus* (Comstock), Alvarez and Van Driesche (1998a) found the larval stage of *C. nipponicus* had a correspondingly high 77% mortality rate.

Certain aspects of the biology and life history of other *Cybocephalus* species have been studied, typically as part of biological control projects, e.g., *Cybocephalus rufifrons* Reitter (DeMarzo 1995), *Cybocephalus freyi* Endrödy-Younga (Lupi 2003), and *Cybocephalus fordori* Endrödy-Younga (Katsoyannos 1984) have been studied in Europe; and *Cybocephalus semiflavus* Champion (Ahmad 1970) and *Cybocephalus gibbulus* Erichson (Nohara and Iwata 1988) have been studied in Asia. In the Middle East, several studies have been carried out on *C. aegyptiacus, C. binotatus, C. micans,* and *C. n. nigriceps* (Blumberg 1973, 1976; Blumberg and Swirski 1974 a, b, 1982). In Australia, Kirejtshuk et al. (1997) described *Cybocephalus aleyrodephagus* and studied its life cycle. The life histories of these species do not differ dramatically from *C. nipponicus,* and there seems to be

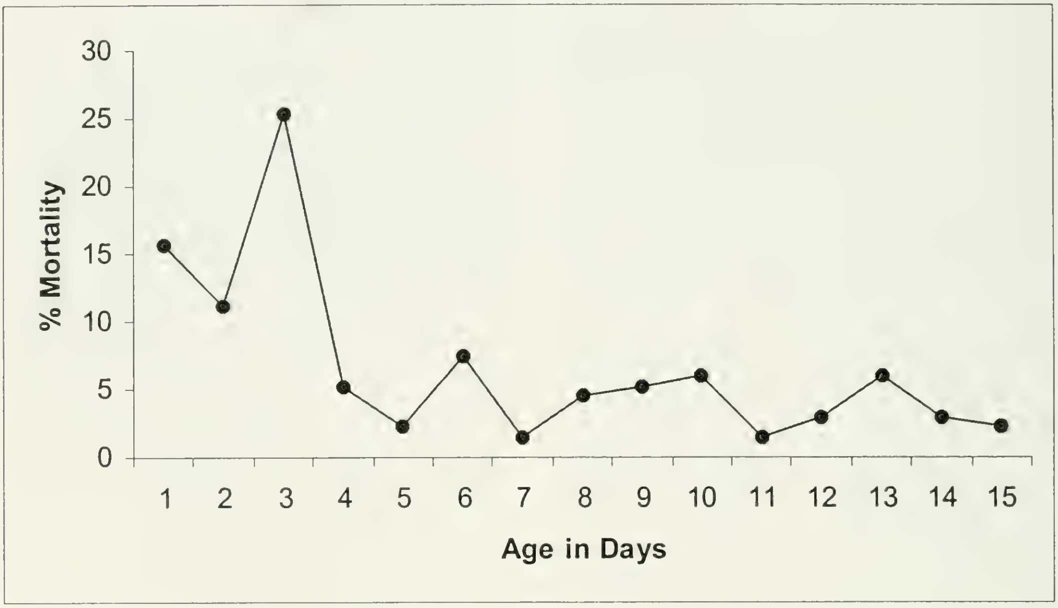


Fig. 9. Larval mortality of *Cybocephalus nipponicus* over time.

fairly consistent life cycle and feeding habits.

In the absence of prey, female cybocephalids are able to withhold eggs for up to 2 days, indicating that oviposition strategy is not only governed by food consumption but also by qualitative features of the scale population (Alvarez and Van Driesche 1998b). In the presence of high scale density, cybocephalids increase egg production until an asymptote is reached. Again, this allows cybocephalids to maintain their populations at low scale densities. If circumstances allow, female cybocephalids will lay only one egg under a single scale cover. However, if the number of scales in a patch is low, and the beetle cannot find new scales, it will lay eggs under a scale under which eggs are already present (Alvarez and Van Driesche 1998b). The beetle larvae are forced to consume more prey when feeding on younger scales, therefore for greater offspring survivability female cybocephalids prefer oviposition on older scales that provide a larger food source and require less searching (Alvarez and Van Driesche 1998b). Alvarez and Van Driesche (1998b) found that the highest larval survival rate could be found in larvae feeding on scales older than 30 days. Blumberg and Swirski (1982) noted that *C. n. nigriceps* rarely laid eggs under dead female scales.

Evans et al. (2005) described two species of *Aphanogmus* (Ceraphronidae) that parasitize *C. nipponicus* pupae. *Aphanogmus inamicus* Evans and Dessart emerged in quarantine from hosts collected in Thailand, and *A. albicoxalis* occurs naturally in Florida. The latter species is broadly distributed in southern Florida, with collection sites in Collier, Miami-Dade, and St. Lucie counties. The levels of parasitism in *C. nipponicus* populations in Florida are currently undetermined, but large numbers of these wasps occasionally have been seen on cycads.

Cybocephalus nipponicus probably always will be considered a supplementary predator for the control of CAS. However, some attributes make these beetles very attractive as biological control agents, including a long lifespan, some ability to resist or protect themselves from pesticides (Alvarez and Van Driesche 1999a, Katsoyannos 1984, Kehat et al. 1974), and most importantly, the ability to persist at low scale densities (Alvarez and Van Driesche 1998b). However, even in combination with the parasitoid *Coccobius fulvus* Compere and Annecke (Hodges et al. 2003) these beetles are unable to adequately control populations of CAS in Florida.

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